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MORPHOLOGIC AND IMMUNOHISTOCHEMICAL STUDIES OF THE PATHOGENESIS OF INFECTION AND ANTIBODY FORMATION SUBSEQUENT TO VACCINATION OF MACACA IRUS WITH AN ATTENUATED STRAIN OF PASTEURELLA TULARENSIS

II. Aerogenic Vaccination

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The information regarding the morbid changes induced by aerogenic vaccination is limited. Gefen and Gordon [1] have studied the morphologic alterations in aerogenically vaccinated guinea pigs, sheep and monkeys. Most of these animals received polyvalent vaccines containing attenuated strains of *Pasteurella tularensis, Bacillus anthracis, Pasteurella pestis,* and *Brucella melitensis.* In a few studies monovalent vaccines were used.

This investigation was carried out in order to define the site and extent of the pneumonic and systemic involvement following aerogenic vaccination against tularemia and to detect differences between dermal and aerogenic vaccinees.

Material and Methods

The preparation and assay of the live vaccine strain of *P. tularensis* (LVS) and the conditioning, housing and feeding of 24 monkeys, *Macaca irus,* used in this study were described in our first paper.[2] These monkeys were exposed to an aerosol of particles, less than 5 μ in diameter, containing LVS. An average inhaled dose of 270,000 viable cells was calculated for the entire group. The larger monkeys received doses as high as 418,000, and the smaller as low as 138,000. The sacrifice schedule, collection of tissues, culture, methods of fixation, processing, staining and observation were the same as those we have reported.[2] In addition, the lobes of the left lung were serially blocked and approximately 40 microsections of lung were examined from each animal.

Results

Cultural Recovery of LVS

LVS was present in the lungs through the 14th day. The initial recovery from the entire lung, at less than one hour after exposure to the aerosol, was 50,000 bacteria. This represents a 20 per cent retention of

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the calculated inhaled dose in these two monkeys. LVS multiplied and a maximum of 3 million was recovered on the third day. Thereafter, the numbers of bacteria gradually diminished in the lungs. One hundred bacteria were present on the 14th day, and none were found on the 28th day. The deep cervical nodes contained organisms on the third through tenth days and the tracheobronchial lymph nodes through the 28th day. LVS was in the spleen on the third, fifth, and 14th days; the highest concentration, 800, occurred on the third day. Bacteria were never cultured from the blood or from inguinal and axillary lymph nodes.

Gross Observations

We saw changes only in the lung and tracheobronchial lymph nodes. Through the fifth day the lungs appeared normal. On the seventh day, distinct punctate areas of reddened parenchyma, 1 to 2 mm. in diameter, were seen randomly distributed throughout all lobes. They were most readily detected through the pleura after the lungs were gently inflated with formalin. In order to determine if these lesions could be detected roentgenographically, 2 monkeys were examined on the seventh day. No abnormalities were observed in the roentgenograms. The lesions persisted, although in diminished numbers, through the tenth day. On the 14th day none were found, and the lungs appeared normal. The tracheobronchial lymph nodes were distinctly enlarged on the fifth day and reached their greatest size, 1 cm., by the tenth to 14th days. No necrosis was found, although numerous petechiae were seen during the second week. This lymphadenitis gradually subsided, and on the 28th day no abnormalities were seen.

Microscopic Observations

At 1, 6, 12, and 24 hours LVS cells * were found in small numbers in the lungs (Fig. 5). When anatomic features were well defined, the bacteria could be located with certainty in the respiratory bronchioles and atrial ducts. No inflammatory exudate was seen on the first day. On the second day a very few (approximately 1 per microsection) minute aggregates of monocytes and a few neutrophils were found along the walls of the respiratory bronchioles (Fig. 1). On the third day these foci were more frequent (3 to 6 per microsection), and the numbers of inflammatory cells had increased and spread proximally. LVS cells were identified in such inflammatory aggregates.

By the fifth day, the inflammatory reaction and LVS involved the rudimentary alveoli arising from the respiratory bronchioles. A distinct

* All references to LVS in this description are based on observations of specifically stained bacteria seen with the fluorescence microscope.

though mild lymphangitis was seen at this time in the peribronchial and perivascular lymphoid tissue. A very few minute mucosal ulcers were found in the proximal portions of respiratory bronchioles. On the seventh day minimal inflammation of the alveoli adjacent to the foci of respiratory bronchiolitis was seen (Figs. 2 and 3). Small patches of fibrinous pleuritis were found where these inflammatory foci were close to the pleura. The peribronchial lymphangitis merged into a lymphocytic and monocytic hyperplasia. By the tenth day resolution of the inflammation was distinct. Monocytes and macrophages were the dominant cells, and neutrophils were difficult to find. The peribronchial lymphoid hyperplasia was still apparent, and plasma cell precursors were found in it. On the 14th day the inflammatory cells in the respiratory bronchioles had disappeared and no stigmas remained, save a minimal increase of macrophages in the interstitial tissues surrounding the arteries entering secondary lobules. The lungs appeared normal, with the exception of an increase of plasma cells in the lymphoid tissue on the 28th day. On the 90th day we could find no residuals.

The microscopic lesions in the tracheobronchial lymph nodes were similar to those described in the axillary lymph nodes previously,[3] but were slightly more intense. The changes in the spleen, liver, and other sites were comparable to those seen in the dermal vaccinees. LVS cells were not seen in tissues other than the lung.

Intracellular anti-tularensis gamma globulin (ATGG) was found in the lung on the seventh day. The plasmocytic cells containing antibody were not numerous. They were located in the interstitial tissue adjacent to the respiratory bronchioles (Fig. 6). At 10 days, the number of antibody-containing cells had increased. In addition, groups of plasma cells containing ATGG were found in the peribronchial and perivascular lymphoid aggregates (Figs. 7 and 8). Intracellular ATGG was found in the tracheobronchial nodes on the fifth day (Fig. 9). The plasmocytic cells that contained antibody were limited to the medullary region of the nodes (Fig. 4). The splenic pulp contained cells with ATGG in their cytoplasm on the fifth day (Fig. 10). The ATGG-containing cells increased in number through the 28th day. ATGG persisted in these sites through the 90th day.

Discussion

The respiratory bronchiole is the site of deposition and of the initial inflammatory response for both LVS and virulent SCHU S4 strains of *P. tularensis*. Whereas SCHU S4 evokes an acute and progressive inflammatory response associated with necrosis, the response to LVS was chiefly monocytic, limited, and without necrosis. In the sites of secondary infection by LVS—the tracheobronchial lymph nodes and spleen—the in-

flammatory response was minimal and transient. This was in contrast to the results of Gefen and Gordon,[1] who found epithelioid cell granulomas in the lymph nodes, spleen, liver and lungs of guinea pigs vaccinated aerogenically with an attenuated Russian vaccine of *P. tularensis*. However, the doses they used were much higher and the guinea pig is less resistant to tularemia than the monkey.

Cellular ATGG appeared sooner in the lung than at the site of dermal vaccination. We assume that this was due to the presence of fewer LVS cells at each focus of respiratory bronchiolitis and that enough ATGG was formed so that all of it was not bound immediately. In the tracheobronchial lymph nodes, spleen and liver, the position, type and number of cells involved in ATGG production were similar to those in the dermal vaccinees. ATGG persisted in the lymphoid tissues of the lung, the spleen, and the tracheobronchial lymph nodes to the 90th day.

The sensitization of many respiratory bronchioles, their associated lymphoid aggregates and the remaining pulmonic lymphoid tissues, with the appearance and persistence of ATGG in these sites, were the only morphologic differences between the aerogenic and dermal vaccinees. This may have significance should aerogenic vaccinees have greater resistance to respiratory challenge.

Summary

Twenty-four cynomolgus monkeys were vaccinated aerogenically with the living vaccine strain (LVS) of *P. tularensis*. The average inhaled dose was 270,000 viable cells. The bacteria initiated a mild, nongranulomatous inflammatory response in the respiratory bronchioles that was completely resolved by the 14th day after vaccination. LVS disseminated to involve the intrapulmonic lymphoid tissues, the tracheobronchial lymph nodes, the liver and the spleen. By the 28th day all sites except the tracheobronchial lymph nodes were sterile, and no bacteria were recovered from these nodes on the 90th day. Anti-tularensis gamma globulin (ATGG) appeared in plasma cell precursors in the lung, about respiratory bronchioles, and in the peribronchial lymphoid tissues by the seventh day. By the 14th day mature plasma cells containing ATGG were prominent. The appearance of ATGG in the regional lymph nodes and spleen was as prompt as that found in the dermal vaccinees.

References

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2. McGavran, M. H.; White, J. D.; Eigelsbach, H. T., and Kerpsack, R. W. Morphologic and immunohistochemical studies of the pathogenesis of infection and antibody formation subsequent to vaccination of *Macaca irus* with an attenuated strain of *Pasteurella tularensis*. I. Intracutaneous vaccination. *Am. J. Path.*, 1961, 41, 259–271.

[Illustrations follow]

Legends for Figures

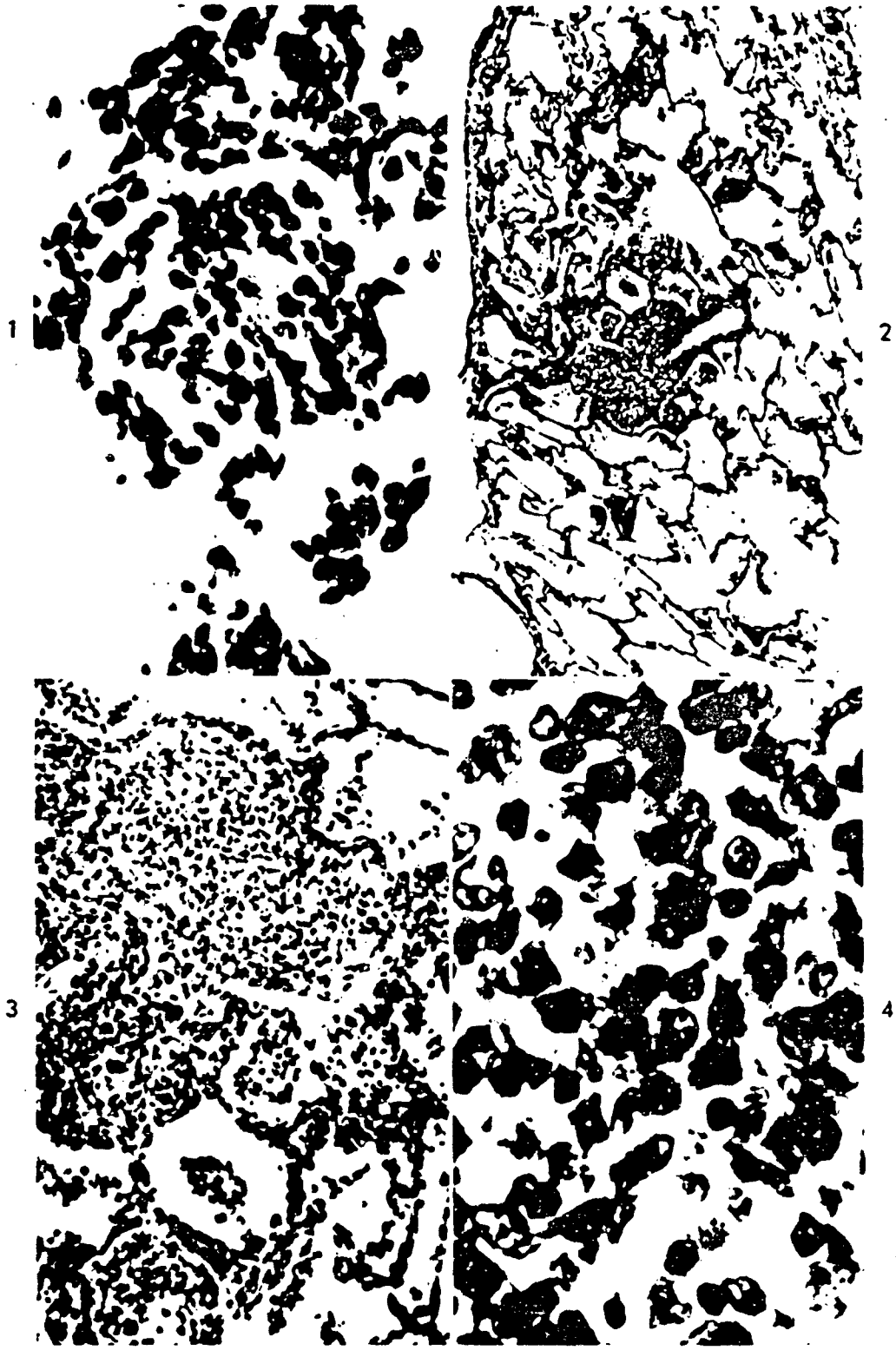
All photomicrographs are of hematoxylin and eosin-stained preparations.

Fig. 1. A rudimentary alveolus in the wall of a respiratory bronchiole is filled with monocytes, lymphocytes and a few neutrophils. This is the extent of the inflammatory reaction 72 hours after aerogenic vaccination. × 600.

Fig. 2. The location and extent of the bronchiolitis on the seventh post-vaccinal day. The adjacent pleura is minimally inflamed. × 60.

Fig. 3. Detail from Figure 2. The inflammatory reaction has spread to involve the adjacent alveoli and the lymphoid sheath about the pulmonary artery. No necrosis of parenchyma is seen. × 195.

Fig. 4. Plasma cells in the medulla of a tracheobronchial lymph node 28 days after aerogenic vaccination. × 600.



All photomicrographs are of fluorescent antibody-stained preparations.

FIG. 5. LVS cells in the wall of a rudimentary alveolus. Section from the lung of a monkey killed 48 hours after aerogenic vaccination. × 100.

FIG. 6. A plasmocyte contains ATGG in the lung on the seventh post-vaccinal day. The cell is located in the wall of a respiratory bronchiole. × 100.

FIG. 7. ATGG-containing cells in peribronchial lymphoid tissue on the tenth post-vaccinal day. Numerous plasma cells are present. × 100.

FIG. 8. Plasma cells in perivascular tissue of the lung contain ATGG 10 days after aerogenic vaccination. × 100.

FIG. 9. An ATGG-containing cell in the tracheobronchial lymph node 5 days after aerogenic vaccination. × 380.

FIG. 10. Cells in the splenic pulp contain ATGG 5 days after aerogenic vaccination. × 100.